1982

Physiology of Salt Excretion in the Mangrove Avicennia marina (Forsk.) Vierh

Philippa M. Drennan
Loyola Marymount University, philippa.drennan@lmu.edu

New Phytol. (1982) **91**, 597–606

PHYSIOLOGY OF SALT EXCRETION IN THE MANGROVE *AVICENNIA MARINA* (FORSK.) VIERH

BY PHILIPPA DRENNAN AND N. W. PAMMENTER

Department of Biological Sciences, University of Natal, Durban, South Africa

(*Accepted 15 March 1982*)

SUMMARY

Diurnal and long-term excretion by leaves of *Avicennia marina* seedlings growing in aqueous culture was correlated with substrate salinity and transpiration. Excretion was greater in 100% than 50% seawater but the reverse was true for transpiration. The diurnal excretion pattern, with exudation minimal during the day and maximal during the night, showed a negative correlation with the daily transpiration pattern. The total amount of salt excreted, however, showed a positive correlation with the total amount of water transpired. Root and xylem sap salinities were linearly related to substrate salinity but leaf Na^+ increased to a maximum, indicating that control of leaf salt content is at the foliar, rather than the root level.

INTRODUCTION

The mangrove species may be divided into two broad groups on the basis of their salt resistance mechanisms: those which exclude salt at the root level, and those which accumulate salt (Levitt, 1972). *Avicennia marina* (Forsk.) Vierh, characteristic of a salt-tolerant species, has a relatively high concentration of salt in the xylem sap (approximately 34 mM; Scholander *et al.*, 1966) and excess salt is removed from the plant by means of excretion at the leaf surfaces (Scholander *et al.*, 1962). Scholander (1968) has shown that exclusion of salt at the root level is a process not directly dependent on respiratory metabolism; a phenomenon which he has termed ultrafiltration. He also found a similar filtering system in the leaves, the continued functioning of which depended only on the integrity of the semi-permeable membranes. It has generally been accepted that excretion in *A. marina* is mediated by the glandular structures on the leaf surfaces (e.g. Shimony, Fahn and Rheinhold, 1973). However, only the young expanding leaves of this species possess glands; as the leaf matures, these glands degenerate (Drennan and Berjak, 1979). There is circumstantial evidence to suggest that in the mature leaf excretion occurs by the movement of liquid water through the stomata (Berjak, 1978; Drennan and Berjak, 1979).

Many physiological parameters have been measured in *A. marina*, e.g. osmotic potentials and transpiration rates (Walter and Steiner, 1936), transpiration rhythms (Steinke, 1979), xylem sap salinities (Scholander *et al.*, 1966), and salt excretion rates and rhythms (Scholander *et al.*, 1962). However, most of these studies have been conducted in the field rather than under controlled conditions. As such the ecophysiological significance of the excretion process is uncertain. There have been no reports of excretion rates or patterns under conditions of controlled substrate salinities. Thus this study centred on the effect of substrate salinity on the physiology of salt excretion.

0028-646X/82/080597+10 $03.00/0

MATERIALS AND METHODS

Plant material

Freshly fallen propagules, collected in the field, were germinated on vermiculite moistened with distilled water. Subsequent to the emergence of the first pair of leaves, the distilled water was supplemented monthly with a modified Hoaglands' nutrient solution (Epstein, 1972). Plants were maintained in a greenhouse. These growth conditions produced a low salt plant, the leaf salt content of which never exceeded 20% of that for equivalent leaves in the field. Seedlings remained in vermiculite until they had at least two pairs of fully mature leaves, at which stage they were used in the various experiments. Experimental sampling of material for leaf salt determinations was restricted to mature leaves, as preliminary experiments indicated that the salt content of young leaves, while consistently higher than that of mature leaves, varied within wide limits.

Methods

Culture procedures. Throughout the various experiments plants were maintained in culture solutions (after Conner, 1969) in a greenhouse. All plants were grown in full-strength nutrient (Epstein, 1972) for 10 days after transfer from vermiculite, and the growth of controlled plants was continued in this solution. During experimental runs plants were grown at salinities over the range 0 to 100% seawater, these being attained by the use of different proportions of seawater: distilled water when preparing culture solutions.

Experimental procedures. To determine the rate of salt excretion, aerial portions of the plant were immersed, at regular intervals, in a known volume of distilled water and agitated for 30 s. The Na^+ concentration of the resulting solution was determined using flame photometry. Flame photometry was also used to determine the Na^+ content of solutions resulting from the wet digest (Jeffries, 1973) of washed, oven-dried root and shoot material. Xylem sap was extruded from a cut stem by means of a pressure chamber, this technique also being used to determine the water potential of the aerial parts of the plant (Scholander *et al.*, 1966). Transpiration was measured as weight loss from a sealed container.

RESULTS

Excretion and transpiration rhythms in A. marina

The long-term pattern of excretion, as monitored daily over a 2-month period, showed no endogenous lunar or tidal rhythm (Fig. 1). Similarly, over the same time period, there was no regular pattern in transpiration rates (Fig. 2). Furthermore, daily transpiration rates did not correlate with daily excretion rates, as would be expected if the transpiration stream was the direct source of excreted salt.

On a diurnal basis there was a definite pattern of excretion showing a midnight maximum and a midday minimum (Fig. 3). However, in heavily excreting plants, or when the weather conditions were cool and overcast, the pattern was less marked. The daily transpiration rhythm was found to be similar to that described by Steinke (1979), with a midday maximum declining to a minimum just before dawn. Concomitant with the daytime increase in transpiration was a decrease in the leaf water potential, a trend reversed when transpiration diminished.

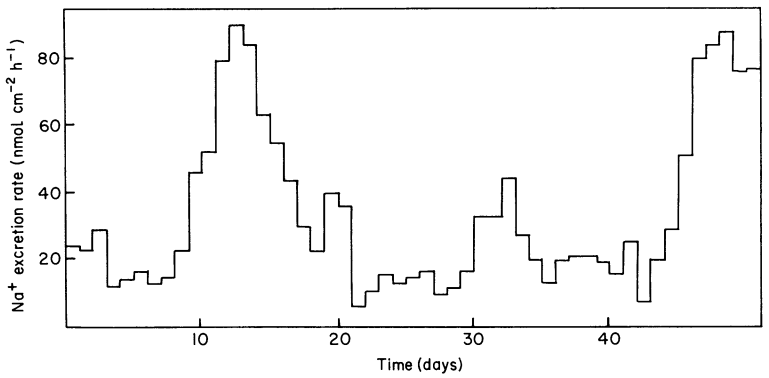

Fig. 1. Long-term pattern of Na^+ excretion of a plant in a 100% seawater culture solution. Measurements were made on plants, each with a minimum of three mature leaf pairs, which had been maintained in 100% seawater for 1 month prior to determining the excretion pattern. The observed differences in rate are thought to reflect the uncontrolled environmental conditions in the greenhouse.

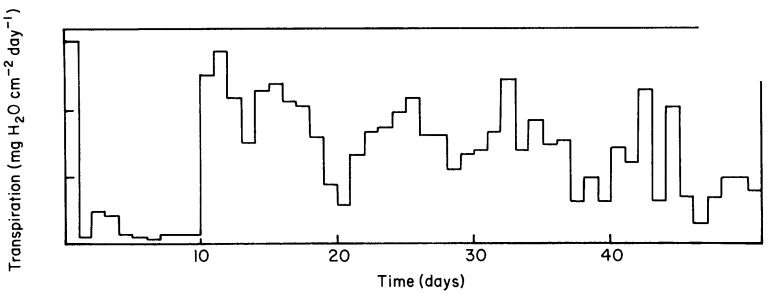

Fig. 2. Long-term pattern in transpiration rates of a plant in a 100% seawater culture solution. As with the excretion pattern, the observed differences in rate are thought to reflect the uncontrolled environmental conditions in the greenhouse.

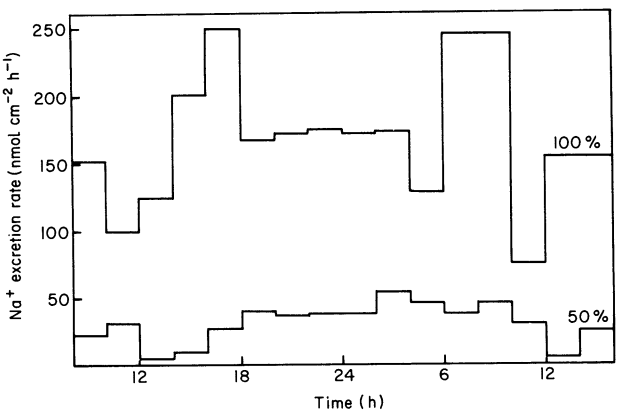

Fig. 3. Diurnal pattern of Na^+ excretion of one plant in 100% and one in 50% seawater culture solution. Most plants showed this diurnal excretion pattern, although in heavily excreting plants, or when weather conditions were cool and overcast, a midday minimum did not occur.

Onset of excretion in non-excreting plants

To determine some of the physiological characteristics associated with the onset of excretion, non-excreting (low salt) plants were transferred into either a nutrient solution, 50% seawater solution or 100% seawater solution. Excretion and transpiration rates were monitored daily. Each day during the first 2 weeks of the experiment, and thereafter at 3-day intervals, a plant from each treatment was sampled to determine water potential and leaf salt content. After 1 month the remaining plants were transferred into non-saline nutrient solution and the resulting changes in the excretory pattern were monitored over the following 4 days.

The mangroves began to excrete within 12 h of being transferred into the saline solutions. The rate of excretion increased for 8 to 10 days after which it remained relatively constant, with the plants in 100% seawater having a slightly higher excretion rate than those in 50% seawater (Fig. 4). Leaf salt content showed a

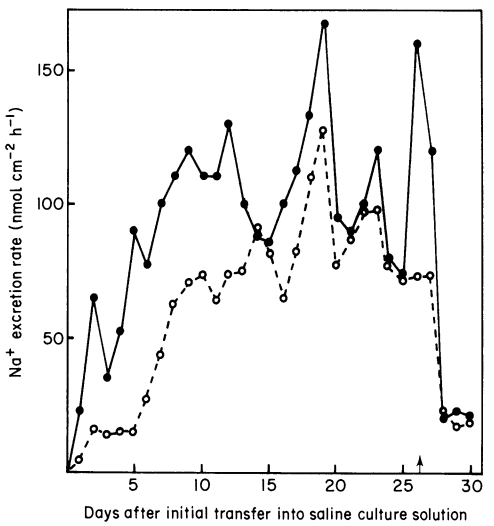

Fig. 4. Average daily Na^+ excretion rate of plants transferred from a freshwater nutrient culture solution to either a 50% (---) or a 100% seawater culture solution (——). Remaining plants were transferred back into freshwater nutrient culture solution on day 26 (arrow).

similar pattern of change, although in this instance this difference between 50 and 100% seawater plants was not significant, both sets of plants having approximately 1·4 (±0·3) mmol Na^+ g^{-1} dry wt (Fig. 5). Transfer into 100% seawater culture solution brought about rapid changes in leaf water potential, which within 24 h dropped below minus 3·8 MPa, some 1·8 MPa lower than the average value for the controls (minus 2·0±0·5 MPa). There was no significant decrease in the water potential of the plants in 50% seawater solutions (minus 2·2±0·5 MPa). The approximate concentration of salt in the leaf was calculated using the known leaf salt content, dry wt and a water content value of 70% (Walter and Steiner, 1936). From those concentrations the contribution of NaCl to the leaf osmotic potential of plants grown at various substrate salinities was calculated (Lang, 1967; Table 1).

The above results suggest that only those plants grown in 50% seawater had

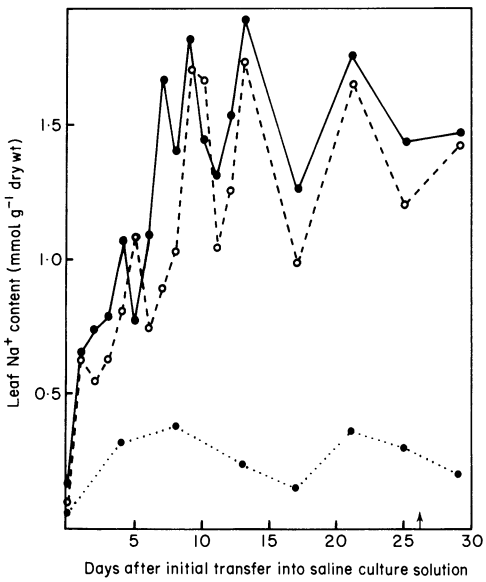

Fig. 5. Leaf Na^+ content following transfer of plants from a nutrient culture solution (·····) to either a 50% (– – –) or a 100% seawater culture solution (——). Remaining plants were transferred back into pure nutrient culture solution on day 26 (arrow).

Table 1. *Measured water potential* (MPa) *and water potential as calculated from the average leaf salt content*

	Salinity of culture medium		
	0% seawater	50% seawater	100% seawater
Measured water potential	$-2{\cdot}0 \pm 0{\cdot}5$	$-2{\cdot}2 \pm 0{\cdot}5$	$-4{\cdot}0 \pm 0{\cdot}6$
Calculated osmotic potential	$-0{\cdot}9$	$-2{\cdot}6$	$-3{\cdot}0$

sufficient salt in their leaves to bring about the measured reduction in water potential. Additional mechanisms for the lowering of leaf water potential must therefore have been operating in both the control plants and those in 100% seawater solutions.

There was little pattern in the transpiration rates for any given set of plants over the experimental period. However, the salinity of the substrate affected the rate of transpiration. As Walter and Steiner (1936) noted, those plants grown in the absence of salt had the lowest transpiration rates. However, the transpiration rates of those plants in 50% seawater solutions were consistently higher than those in 100% seawater solutions (Table 2).

Despite the negative correlation diurnally, in the long term the total amount of Na^+ excreted by a plant correlated linearly with the total water loss by transpiration (Fig. 6). Daily transpiration and Na^+ excretion were measured for five plants growing in 50% seawater and four plants growing in 100% seawater for a period of 21 days. The ratio of total Na^+ excreted to total water transpired was calculated for each plant over this 21-day period. The means of 50 and 100% seawater were $32{\cdot}6 \pm 4{\cdot}9$ and $62{\cdot}9 \pm 17{\cdot}7$ μmol Na^+ g^{-1} H_2O respectively. This gives a ratio of this

Table 2. *Hourly transpiration rates of* Avicennia marina *grown at different substrate salinities (as averaged from long-term transpiration data)*

	Substrate salinity		
	0% seawater	50% seawater	100% seawater
Transpiration rate (mg H_2O cm^{-2} h^{-1})	5·0±2·3	15·4±4·5	8·5±4·5

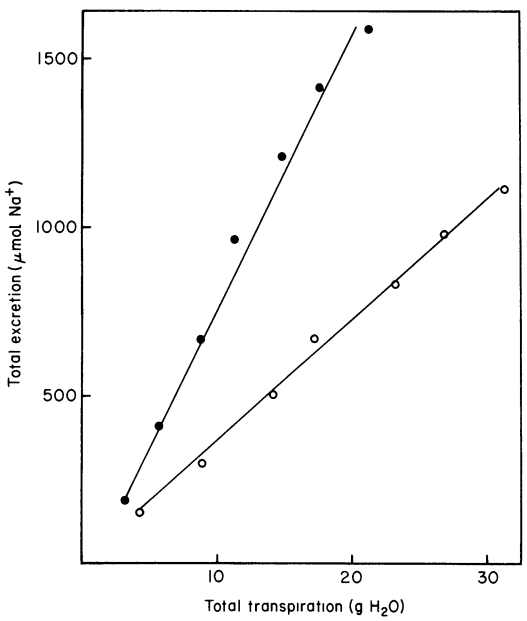

Fig. 6. Relationship between total Na^+ excreted and total transpiration for a plant in each of 50% seawater nutrient solution (○) and 100% seawater nutrient solution (●). Cumulative totals for excretion and transpiration were calculated at 3 day intervals over the 3 week period. Plants were maintained in the appropriate solutions for 1 month prior to measurement. The difference in slope between the two regression lines can probably be attributed to the difference in xylem sap concentration between a plant in 100% seawater culture solution and one in 50% seawater culture solution. The curves are described by the following equations:

100% seawater $E = 80{\cdot}5T - 30{\cdot}5$, $r = 0{\cdot}99$ 50% seawater $E = 36{\cdot}2T + 7{\cdot}0$, $r = 0{\cdot}99$

where E = total excretion and T = total transpiration. The ratio of the slopes 100% seawater: 50% seawater is 2·22. This is similar to the ratio of xylem sap concentrations for these two treatments; xylem sap concentration 100%: xylem sap concentration 50% is 2·01 (refer to Fig. 8).

value between 100 and 50% seawater of 1·93, which is similar to the ratio of the xylem sap salinities of these two treatments at a value of 2·01 (see Fig. 8). This indicates that the salt reaches the leaves via the transpiration stream. Assuming that all the salt transported in the xylem sap is excreted by the leaves, these long-term excretion/transpiration measurements indicate xylem sap concentrations of 32·6 and 62·9 mM (μmol Na^+ g^{-1} H_2O) for 50 and 100% seawater respectively. This is lower than the 50 and 100 mM, respectively, measured for xylem sap concentrations. However, these two experiments were performed on different plants at different times of the year, and this could account for the discrepancy.

On return of plants to non-saline nutrient solution, excretion continued unabated for 48 h, after which it dropped to a negligible level. There was no marked change in the other parameters measured, viz. leaf water potential, leaf salt content, and transpiration rates. However, it is probable that had the experiment been continued longer, some change in the values would have been noted.

Effect of substrate salinity on the salt content of the plant

Collation of data from various experiments showed that, over the range 0 to 100 % seawater, there was a linear correlation between root salt content and substrate salinity (Fig. 7), as there was between xylem sap concentration and substrate salinity (Fig. 8). However, a plot of leaf salt content as a function of substrate salinity yielded a classical saturation-type curve (Fig. 9). Although salt contents of leaves, roots and xylem sap varied with substrate salinity, they showed no diurnal variation.

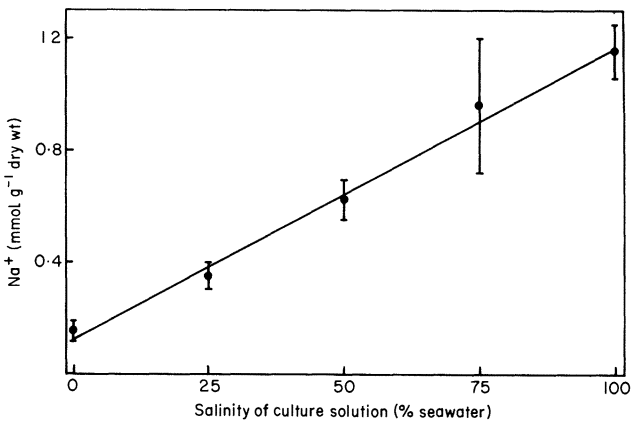

Fig. 7. Relationship between the salinity of the culture solution and root Na^+ content. Vertical bars represent one standard deviation; $r = 0{\cdot}99$.

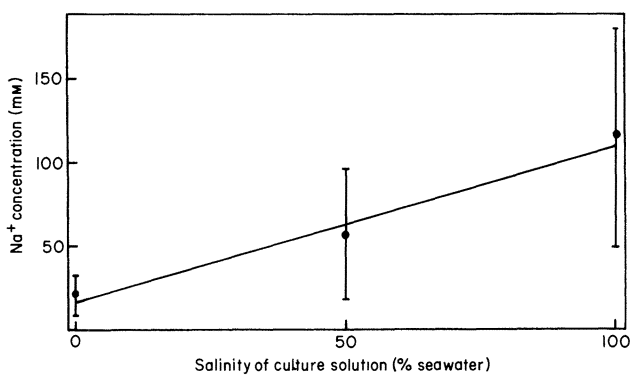

Fig. 8. Relationship between the salinity of the culture solution and xylem sap Na^+ concentration. Vertical bars represent one standard deviation; $r = 0{\cdot}98$.

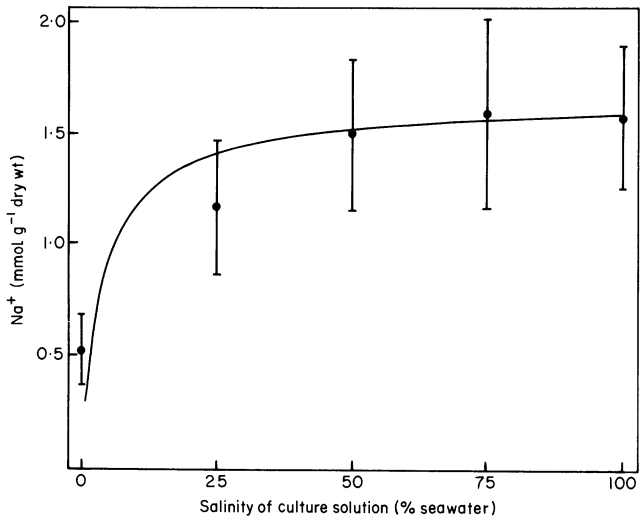

Fig. 9. Relationship between the salinity of the culture solution and leaf Na^+ content. Vertical bars represent one standard deviation; $r = 1{\cdot}00$.

DISCUSSION

Avicennia marina is a typically salt-tolerant species in that there is only partial salt exclusion at the root level, with excess salt being removed from the plant by means of excretion at the leaf surfaces. Wide variations have been obtained when certain physiological parameters of *A. marina*, e.g. osmotic potential of xylem sap, were measured in the field (Scholander *et al.*, 1966). Results of the present study indicated that such variations are related to the salinity of the substrate.

Contrary to the findings of Scholander *et al.* (1962) the diurnal excretory pattern of *A. marina* was not arhythmic, having a midday minimum and a midnight maximum. A light-positive excretory response, as found by that author in the mangroves *Aegiceras* and *Aegialitis*, can be interpreted either in terms of a light-dependent energy mechanism or in terms of an immediate excretion of the salts arriving in the transpiration stream. However, in *A. marina* the lack of correlation between transpiration and excretion on a daily basis and the negative correlation over the diurnal period implied some type of salt storage phenomenon and a degree of separation of the processes of excretion and transpiration. Such a separation suggests that salt arriving at the leaf during daytime transpiration must be sequestered prior to excretion. Continued excretion after removal of salt from the substrate further supports the idea of a storage phenomenon. This storage phenomenon would be further substantiated by a diurnal pattern in leaf salt content corresponding inversely to the excretion pattern. Such a pattern was not apparent despite the short leaf-salt residence time of approximately 1 day. (Using the dry wt per unit area of a leaf, both the salt content per leaf and total Na^+ excretion per leaf per day can be calculated. For plants in 100 % seawater the values for total leaf salt content and total daily excretion are approximately the same, suggesting a leaf residence time of 1 day. For plants in 50 % seawater the leaf residence time would be slightly longer.) As with leaf salt, there were no marked diurnal changes in root salt content and xylem sap salinity, the latter confirmed by Scholander *et al.* (1966). There is, however, preliminary ultrastructural

evidence to support this storage concept (Berjak, 1978). This area requires further study.

Scholander (1968) has shown that Na^+ transport into the root is a non-metabolic phenomenon (ultrafiltration). The work presented here has shown a linear correlation between root salt content and substrate salinity over the range 0 to 100% seawater. This indicates that, whatever the uptake mechanism, it is not saturated at a Na^+ concentration of 450 mM (100% seawater). This contrasted with the typical saturation curve obtained when leaf salt content was plotted as a function of substrate salinity over the same range. This implicated the leaf as the major site of salt control in *A. marina*. Leaf salt content is not directly related to xylem sap salt content, suggesting that leaf salt content is maintained at a given level by the process of excretion and does not reflect the extent of ion exclusion at the root level. Kylin and Gee (1970) have demonstrated *in vitro* an Na^+-stimulated ATPase from the leaves of *Avicennia nitida*, a salt-excreting mangrove. It is possible that a similar system exists in *A. marina* to control the leaf salt content.

Onset of excretion is apparently triggered once a low-threshold leaf salt level has been exceeded. While plants with a leaf salt content as low as 0·65 mmol g^{-1} dry wt excreted salt, the controls, with an average of $0{\cdot}4 \pm 0{\cdot}2$ mmol g^{-1} dry wt, were never found to be excreting. Thus the leaf threshold must lie between these two values. The higher excretion rates observed at increased substrate salinities appeared to act to maintain leaf salt content below a maximum of 1·4 mmol g^{-1} dry wt.

The physiological basis of the effect of substrate salinity on transpiration rate of *A. marina* is unknown. It is interesting to note, however, that transpiration is greatest in plants grown in 50% seawater, the salinity at which Connor (1969) observed maximal growth.

Although showing this optimal salinity growth response, *A. marina* is apparently well adapted to the euryhaline environment, in that over the range 0 to 100% seawater an increase in the excretion rate maintains a constant leaf salt content. This contrasts with the physiology of the less salt-tolerant halophytic grass *Aeluropus littoralis*, In this species, which has an optimum growth at a salinity of approximately 15% seawater (Waisel, 1972), an increase in substrate salinity results in an increase in leaf salt content, while excretion rate peaks at a substrate salinity of 50% seawater (Pollak and Waisel, 1979). Thus in *A. marina*, while further investigation into the ionic composition of excretion is necessary to determine the ecological importance of this phenomenon with respect to nutrition, the excretory physiology of this species reflects its adaptation to the euhalophytic estuarine environment. As it appears that mature, but salt-excreting, leaves do not possess functional glands (Drennan and Berjak, 1979) more work is required on the mechanism of salt excretion by the leaves of this species.

References

Berjak, P. (1978). Subcellular adaptations in plants of extreme environments. II. Towards understanding the salt excretion in *Avicennia* spp. *Proceedings of Electron Microscopy Society of Southern Africa*, **8**, 101–102.

Connor, D. J. (1969). Growth of the grey mangrove *Avicennia marina* in nutrient culture. *Biotropica*, **1**, 36–40.

Drennan, P. & Berjak, P. (1979). Degeneration of foliar glands correlated with the shift to abaxial salt excretion in *Avicennia marina* (Forsk.) Vierh. *Proceedings of Electron Microscopy Society of Southern Africa*, **9**, 83–84.

EPSTEIN, E. L. (1972). *Mineral Nutrition in Plants: Principles and Perspectives*. Wiley, New York.

JEFFRIES, R. L. (1973). The ionic relations of the seedling of the halophyte *Triglochin maritima* L. In: *Ion Transport in Plants* (Ed. by W. P. Anderson), pp. 297–321. Academic Press, London.

KYLIN, A. & GEE, R. (1970). Adenosine triphosphatase activities in the leaves of the mangrove *Avicennia nitida* Jacq. *Plant Physiology*, **45**, 169–172.

LANG, A. R. G. (1967). Osmotic co-efficients and water potentials of sodium chloride solutions from 0 to 40 °C. *Australian Journal of Chemistry*, **20**, 2017–2023.

LEVITT, J. (1972). *Response of Planta to Environmental Stresses*. Academic Press, New York.

POLLAK, G. & WAISEL, Y. (1979). Ecophysiology of salt excretion in *Aeluropus litoralis* (Gramineae). *Physiologia Plantarum*, **47**, 177–184.

SCHOLANDER, P. F. (1968). How mangroves desalinate seawater. *Physiologia Plantarum*, **21**, 251–261.

SCHOLANDER, P. F., HAMMEL, H. T., HEMMINGSON, E. D. & GAREY, W. (1962). Salt balance in mangroves. *Plant Physiology*, **37**, 722–729.

SCHOLANDER, P. F., BRADSTREET, E. A., HAMMEL, H. T. & HEMMINGSON, E. D. (1966). Sap concentrations in halophytes and some other plants. *Plant Physiology*, **41**, 529–532.

SHIMONY, C., FAHN, A. & RHEINHOLD, L. (1973). Ultrastructure and ion gradients in the salt glands of *Avicennia marina* (Forsk.) Vierh. *New Phytologist*, **72**, 27–36.

STEINKE, T. (1979). Apparent transpirational rhythms of *Avicennia marina* (Forsk.) Vierh. at Inhaca Island, Moçambique. *Journal of South African Botany*, **45**, 133–138.

WAISEL, Y. (1972). *Biology of Halophytes*. Academic Press, New York.

WALTER, H. & STEINER, M. (1936). Die ökologie der ostafrikanischen Mangroven. *Zeitschrift fur Botanik*, **30**, 65–193.